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● *Original Contribution*

REPRODUCIBILITY OF BRACHIAL VASCULAR CHANGES WITH ALTERATIONS IN END-TIDAL CARBON DIOXIDE

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Abstract—The purpose of this study was to examine the reproducibility of the peripheral vascular response to hypercapnia. Healthy college-aged men (n = 7) and women (n = 10) underwent an iso-oxic 10-mm Hg increase in $P_{et}CO_2$ for 12 min. Brachial artery diameter changes were measured using ultrasound imaging. Two tests were completed on day 1 with 15 min of rest between tests. Tests were repeated on day 2. Paired *t*-tests, Bland–Altman plots and intra-class correlations (ICCs) determined reproducibility. There were no significant differences in peak dilation within day (5.33 ± 3.73% vs. 4.52 ± 2.49%, $p = 0.378$). The within-day ICC was poor (0.213). Within-day time-to-peak dilation did not significantly differ (660.0 ± 231.8 s vs. 602.7 ± 259.9 s, $p = 0.379$), and the ICC was fair (0.416, $p = 0.113$). Between-day peak dilation did not significantly differ (5.24 ± 3.84% vs. 4.71 ± 3.17%, $p = 0.123$), and the ICC was fair (0.419). Hypercapnia-induced brachial artery dilation is similar within day and between days. The ICC for peak dilation suggests the methodology is not reproducible. (E-mail: denge001@umn.edu) © **2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Hypercapnia, Ultrasound, Vasodilation.**

INTRODUCTION

Almost a quarter of all deaths in the United States are related to heart disease, making it the number one cause of death (Heron 2013). Cardiovascular disease (CVD) is a health concern not only in the United States, but also in the world, where it is also the leading cause of death (Pagidipati and Gaziano, 2013). Given the impact of CVD on mortality worldwide, early identification of the disease has garnered considerable attention. Because of this interest, several invasive and non-invasive methods have been developed to assess both vascular structure and function are associated with difference stages of the development of CVD. A non-invasive approach to measuring endothelium-dependent dilation is high-resolution ultrasound imaging of arterial response to reactive hyperemia (Laurent et al. 2006). This method of using reactive hyperemia to measure endothelium-dependent dilation of the brachial artery is often referred to as flow-mediated dilation (FMD). FMD is affected by both baseline vessel diameter and peak shear rate during the reactive hyperemia-induced post-cuff occlusion (Khairy et al. 2010; Marlatt et al. 2013). In addition, some individuals find the high cuff inflation pressures needed to occlude the artery painful. Therefore, alternative methods that challenge the artery to dilate may prove to be useful in the assessment of vascular function.

Previously, researchers (Ainslie et al. 2005; Blair et al. 1960; Kontos et al. 1972; Vantanajal et al. 2007) have reported that alterations in arterial carbon dioxide volumes (P_aCO_2) *via* inhalation of varying concentrations of carbon dioxide (CO_2) result in vasodilation of the peripheral vasculature in humans. These studies, however, failed to control for concurrent changes in arterial oxygen volumes (P_aO_2), which have also been found to elicit peripheral vasodilatory effects similar to those produced during hypercapnia (Simmons et al. 2007). To accurately describe the independent effects of P_aCO_2 on the peripheral vasculature, P_aO_2 needs to be maintained.

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One way to manipulate P_aCO_2 while maintaining P_aO_2 is end-tidal forcing, a method that independently controls end-tidal carbon dioxide ($P_{et}CO_2$) and end-tidal oxygen ($P_{et}O_2$) (Ainslie et al. 2005; Essfeld et al. 1990; Vantanajal et al. 2007). Ainslie et al. (2005) used end-tidal forcing to examine the peripheral blood flow response to hypercapnia and reported no significant femoral blood flow changes in response to a hypercapnic, euoxic state. Sato et al. (2012) used transcranial Doppler (TCD) and ultrasound to compare blood flow velocities of the middle cerebral artery (MCA) in response to peripheral measures in the external carotid artery, internal carotid artery and vertebral artery. Sato et al. (2012) identified significantly lower blood flow responses to hypercapnia in the peripheral arteries than in the MCA. Other studies have also used the end-tidal forcing method to alter end-tidal gases while examining cerebral vascular reactivity with magnetic resonance imaging (MRI) (Kassner et al. 2010; Mandell et al. 2008; Mark et al. 2010, 2011; Mutch et al. 2012; Prisman et al. 2008). The end-tidal forcing method allows for concurrent control of $P_{et}CO_2$ and $P_{et}O_2$, which enables researchers to isolate the effects of end-tidal gas manipulation on the vasculature (Mandell et al. 2008; Prisman et al. 2008). End-tidal forcing has also been used in conjunction with ultrasound to examine peripheral vascular changes of the carotid arteries and femoral artery during a hypercapnic state (Ainslie et al. 2005; Sato et al. 2012). Despite this previous work, to date, the reproducibility of end-tidal forcing for examination of the manipulation of various peripheral arterial beds has yet to be determined. Therefore, the purpose of this study was to examine the feasibility and reproducibility of controlling end-tidal $P_{et}CO_2$ and $P_{et}O_2$ for the assessment of peripheral vascular function.

METHODS

Patients

The University of Minnesota institutional review board approved this study. Thirty participants were recruited to participate in the study at the University of Minnesota, and consented to participation. Five participants were unable to perform the breathing protocol (the participants felt uncomfortably short of breath) and withdrew from the study. The remaining 25 participants (mean age: 22.9 ± 3.0 y) were free of neurologic disorders and drug or alcohol dependence. Participants were required to abstain from caffeine consumption and exercise 12 h before participation. Participants were also required to fast for 6 h before participation to eliminate the potential effects of diet on the vasculature.

Of the 25 participants, 17 completed all phases of testing. The forced, deep respiratory responses required for the breathing challenge created a great deal of movement during ultrasound image acquisition. This movement artifact resulted in poor image capture resolution during ultrasound imaging for some participants. In these cases, participant scans were not used, contributing the significant dropout rate.

Gas manipulation and vascular imaging

All testing was performed in the University of Minnesota's Laboratory of Integrative Human Physiology. Patients were tested in a quiet, climate-controlled room (22°C–23°C). Measurements of height and weight were obtained with a standard digital scale and stadiometer (Detecto, Webb City, MO, USA). Body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in meters squared (m^2). Participants were placed in the supine position with the left arm extended and supported. A specially designed re-breathing mask was fitted, taped to the participant's face to prevent air leaks and connected to a sequential gas delivery breathing circuit (Thornhill Research, Toronto, ON, Canada). A computer-controlled end-tidal forcing device (RespirAct, Thornhill Research) was used to manipulate $P_{et}CO_2$ and $P_{et}O_2$ values. Baseline $P_{et}CO_2$ and $P_{et}O_2$ values were obtained with the participant resting in the supine position before the study.

An audio-based metronome (Thornhill Research) was used to help participants maintain the targeted breathing rate of 12 breaths/min. $P_{et}O_2$ values were targeted to resting values throughout the protocol to ensure an iso-oxic environment was maintained. Baseline $P_{et}CO_2$ values were initially maintained for a period of 2 min and were then increased 10 mm Hg from baseline for a period of 12 min (Fig. 1). After the period of elevated $P_{et}CO_2$ values, $P_{et}CO_2$ was returned to baseline for a period of 1 min. Ultrasound imaging of the brachial

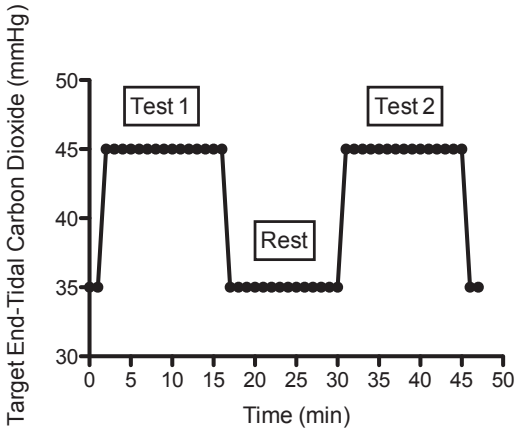

Fig. 1. Time course of targeted end-tidal carbon dioxide volumes for tests 1 and 2.